

IS TUMOR NECROSIS FACTOR - 376A PROMOTER POLYMORPHISM ASSOCIATED WITH SUSCEPTIBILITY TO MULTIPLE SCLEROSIS?

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Abstract A single nucleotide polymorphism (SNP) at position -376 of the tumor necrosis factor α gene (TNFA) has been associated with susceptibility to multiple sclerosis (MS) in Spain. However, no association was found in populations from the USA and The Netherlands. Here we investigate the association between the TNFA - 376A SNP and MS susceptibility in Argentinean patients with MS. The A/G genotype was found in 4.4% of patients (n=90) and in 4.8% of healthy individuals (n=84; p=0.92; odds ratio=0.93; confidence interval: 0.23-3.84). Thus, no significant differences in genotype and allele frequencies were found between healthy individuals and patients with MS in Argentina.

Key words: genetic risk; multiple sclerosis; single nucleotide polymorphism; tumour necrosis factor

Resumen ¿El polimorfismo-376A del promotor del gen del factor de necrosis tumoral se asocia con una mayor susceptibilidad a padecer esclerosis múltiple? Un polimorfismo de nucleótido único (SNP, por sus iniciales en inglés) en la posición -376 del gen codificante del factor de necrosis tumoral α (TNFA) ha sido asociado en España con un mayor riesgo a padecer esclerosis múltiple (EM). Sin embargo, esta asociación no fue encontrada en estudios hechos en poblaciones provenientes de los EE.UU. y Holanda. Aquí investigamos la asociación entre el SNP TNFA -376A y el desarrollo de EM en una población de pacientes argentinos con EM. El genotipo A/G fue encontrado en 4.4% de los pacientes (n=90) y en 4.8% de los controles sanos (n=84; p=0.92; odds ratio=0.93; intervalo de confianza: 0.23-3.84). En consecuencia, no encontramos diferencias en las frecuencias alélicas y genotípicas entre los sujetos enfermos y los controles sanos en Argentina.

Palabras clave: riesgo genético, esclerosis múltiple, polimorfismo de nucleótido único, factor de necrosis tumoral

Multiple sclerosis (MS) is an autoimmune disease initiated by major histocompatibility complex (MHC) class II-restricted CD4+ T lymphocytes, which produce pro-inflammatory helper type 1 cytokines such as interferon- γ , interleukin-2, tumor necrosis factor α and lymphotoxin. Epidemiological evidence indicates that MS results from unidentified environmental agents acting on genetically susceptible individuals¹.

MS is a complex genetic disorder in which only alleles of the MHC have reproducibly been associated with the disease in molecular epidemiology studies. There have been several initial reports indicating a positive association between polymorphisms in different genes and MS but these results could not be replicated in independent populations².

A single nucleotide polymorphism (SNP) at position -376 of the promoter region of the tumor necrosis factor α gene (TNFA) was associated with susceptibility to MS in Spain^{3,4}. However, this association could not be reproduced in two studies performed in populations from Rochester, USA⁵ and Amsterdam, The Netherlands⁶ suggesting that this polymorphism might be population-specific. Thus, an admixed population is well suited to explore this question further.

The polymorphism analyzed here consists of a guanine (G) to adenine (A) transition. This nucleotide variation, located in a regulatory region of the TNFA gene, could be functionally active. In this line, Knight et al. reported that the G to A transition affects the binding of the OTC-1 transcription factor to the promoter, thereby modulating the transcription rate of the TNFA gene⁷. However, these findings could not be replicated by others⁸. Another point of interest is that there are important differences in the population frequency of this polymorphism. The TNFA -376 SNP is particularly frequent in inhabitants of Southern Europe⁹.

Received: 19-IV-2007

Accepted: 21-VI-2007

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The aim of the present study was to examine the association between the TNFA-376A SNP and MS susceptibility in an admixed population of Argentinean patients with MS.

Materials and Methods

Study population

We studied a cohort of 90 Argentinean patients with relapsing remitting MS who were consecutively recruited at the MS clinic of Ramos Mejía Hospital, Buenos Aires, Argentina, from March 2005 to January 2006. The study was reviewed and approved by the local ethical and research committee and written informed consent from each patient was obtained prior to any sample recovery. All patients underwent a standard battery of examinations, including medical history, physical and neurological examinations, screening laboratory tests and brain magnetic resonance imaging. Diagnosis was made in accordance with the McDonald criteria¹⁰. Clinical and demographic data collected included family history of MS and ethnic origin of first- and second- degree relatives. To compare this study population with those in which the TNFA -376A SNP association was found in previous studies, particular interest was paid to those patients whose family members originated from Spain. The control group comprised 84 non-related healthy individuals who were matched for ethnic background, gender and age, without positive antecedents of neurological, autoimmune or psychiatric disease.

TNFA-376A SNP genotyping

Genomic DNA was isolated from whole blood using a Flexigene kit, as described by the manufacturer (Qiagen, Hilden, Germany). The presence of the TNFA -376A SNP was assessed blind to clinical status by polymerase chain reaction (PCR)-restriction fragment length polymorphism assay using oligonucleotide primers: 5'TCTCGGTTTCTTCCATCG and 5' GAGTCTCCGGTCAGAATGA. Annealing conditions were at 60 °C for 150 seconds and 35 cycles. A 10 µl sample of PCR product was digested with 4U Tsp509 in a final volume of 20 µl. Allele G yielded two fragments of 51 and 485 base pairs and allele A yielded three fragments of 51, 247 and 238 base pairs. Fragments were resolved by 2% agarose gel electrophoresis.

Statistical analysis

Allele and genotype frequencies were obtained by direct counting. Hardy-Weimberg equilibrium was tested by exact

test and Chi-square was used to test for differences in allele and genotype distribution between groups. The odds ratio was calculated together with 95% confidence intervals from contingency tables. All statistical analyses were performed using SPSS version 10.0 for Windows.

Results

The distribution of genotypes and allele frequencies of the TNFA -376 SNP in patients and controls is summarized in Table 1. The genotype count in the control group was in Hardy-Weimberg equilibrium ($p=1$). The A/G genotype was found in 4.4% of patients with MS and in 4.8% of healthy individuals ($p=0.92$, OR=0.93, CI=0.23-3.84). Thus, no significant differences in genotype and allele frequencies were found between groups of healthy individuals and patients with MS.

Only one patient with MS had a family history of MS. Four of patients had a first –or second– degree Spanish ancestor, one of them being a carrier of the TNFA-376A SNP. Thus, A/G genotypes were not associated with either a family history of MS or with Spanish ancestry.

Discussion

In contrast to both Spanish reports, results of the current study fail to document an association between the TNFA -376A SNP variation and MS susceptibility. Pooled data from these studies show an over-representation of the hypothesized risk allele (-376A) in Spanish patients with MS, with a frequency of 0.165 compared with 0.092 in healthy individuals⁴, whereas in the present study, no significant differences were observed. These results are coincident with the negative findings observed by Weinschenker et al.⁵ and the Jong et al.⁶ in North-American and Dutch populations, respectively.

Failure to replicate the positive association reported previously could be as a result of several factors. Apart from genotyping errors, which are very unlikely, chance is a frequently cited explanation for initial positive findings that cannot be replicated by other researchers¹¹. However, it seems unlikely that the same bias would have

TABLE 1.– Genotypic distributions of the -376A SNP in the TNFA gene in patients with MS

-376A SNPS	Patients with MS (%) (n=90)	Healthy individuals (%) (n=84)	Odds ratio (95% confidence interval)	p value
A/A	0	0	N/A	0.92
A/G	4 (4.4)	4 (4.8)	0.93 (0.23-3.84)	
G/G	86 (95.5)	80 (95.2)	1	

MS, multiple sclerosis; SNP, single nucleotide polymorphism; TNF, tumor necrosis factor. N/A, not applicable
p value estimated from Yates corrected Pearson χ^2 statistic (two degrees of freedom: A/G + A/A vs. G/G comparison).

been influencing both Spanish studies. Population stratification is another frequently cited reason for lack of replication in molecular genetic epidemiology¹², while other authors argue that this factor is of limited importance¹³. Nevertheless, as Martinez et al.⁴ recognized in their report, population stratification as a confounder was not formally excluded. The Spanish study recruited cases and controls from two geographically distinct populations; furthermore, there is evidence that Europeans cannot be divided easily into readily discernable genetic groups, but instead belong to multiple clusters. Thus, population stratification may have been a confounding factor¹⁴.

Population differences in susceptibility alleles or allele heterogeneity might be another reason that the association between the TNFA -376A SNP and susceptibility to MS could not be confirmed¹⁵. This could arise if different disease-causing alleles are predominant in different study populations; a variation in the degree of linkage disequilibrium between marker and disease alleles exists; or allele frequencies are similar, but heterogeneity in the size of the effect of the disease gene between study settings is present. This last possibility could arise when there are extreme differences in the prevalence of environmental factors that could be modifying the genetic effect¹¹. Large variations in the absolute risk of disease between populations under study could indicate that gene-environment interactions might be playing a role in the development of the pathology of the disease. As the frequency of the A allele in both the Spanish population and the Argentinean population is similar, and the prevalence of MS in Spain is two- or three-fold higher than that in Argentina^{16,17}, we suggest that gene-environment interaction could be a potential explanation for our lack of replication.

In summary, the -376A TNFA variant does not seem to be an important genetic risk factor for MS in our population. Our negative result shows the importance of performing replication studies in different populations before the identification of susceptibility alleles to complex disorders could be useful in the clinic, as susceptibility loci might differ in different ethnic groups.

Acknowledgements: This work was supported by grants (to A.M.V) from *Ministerio de Salud y Ambiente de la Nación*, Argentina. M.K. was supported by a clinical research fellowship from *Fundación Florencio Fiorini*. The authors thank the individuals with MS and their families for making this study possible. They also thank Alicia Mizchenko, Paola Barrero, and Valentina Fuse, for helpful comments and technical support.

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